TITLE

Altered effective connectivity contributes to micrographia in patients with Parkinson's disease and freezing of gait

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ABSTRACT

Recently, it was shown that patients with Parkinson's disease (PD) and freezing of gait (FOG) can also experience freezing episodes during handwriting and present writing problems outside these episodes. So far, the neural networks underlying increased handwriting problems in subjects with FOG are unclear. This study used dynamic causal modeling of fMRI data to investigate neural network dynamics underlying freezing-related handwriting problems and how these networks changed in response to visual cues. Twenty-seven non-freezers and 10 freezers performed a pre-writing task with and without visual cues in the scanner with their right hand. The results showed that freezers and non-freezers were able to recruit networks involved in cued and uncued writing in a similar fashion. Whole group analysis also revealed a trend towards altered visuomotor integration in patients with PD. Next, we controlled for differences in disease severity between both patient groups using a sensitivity analysis. For this, a subgroup of 10 non-freezers matched for disease severity was selected by an independent researcher. This analysis further exposed significantly weaker coupling in mostly left hemispheric visuo-parietal, parietal-supplementary motor area, parietal-premotor, and premotor-M1 pathways in freezers compared to non-freezers, irrespective of cues. Correlation analyses revealed that these impairments in connectivity were related to writing amplitude and quality. Taken together, these findings show that freezers have reduced involvement of the supplementary motor area in the motor network, which explains the impaired writing amplitude regulation in this group. In addition, weaker supportive premotor connectivity, may have contributed to micrographia in freezers, a pattern that was independent of cueing.

KEYWORDS

Parkinson's disease; Freezing of gait; Micrographia; Visual cueing; Dynamic causal modeling

INTRODUCTION

One of the most debilitating motor symptoms in Parkinson's disease (PD) is freezing of gait (FOG), i.e. '*a brief episodic absence or marked reduction of forward progression of the feet despite the intention to walk*' [1]. A characteristic of this disturbing symptom is its remarkable responsiveness to visual or auditory cues [1-4]. In the past years, freezing is no longer considered a mere gait problem, as evidence is accumulating that freezing episodes also occur during repetitive upper limb movements or speech [5, 6]. More recently, our group revealed that in part of the patients with PD freezing episodes take place during handwriting-like movements [7] and that freezers experience decreased writing amplitude, i.e. aggravated micrographia, and increased variability outside these freezing episodes [8]. Similar to gait, non-gait freezing is responsive to cueing [9].

Up till now, research revealing the differences in brain networks between freezers and non-freezers has mainly focused on structural and functional connectivity (for a review see [10]), as well as on activation patterns when imagining gait and during actual freezing episodes of the hands and feet [11-13]. In studies on motor imagery of gait, decreases were found in cortical regions, including the supplementary motor area (SMA) and superior parietal area (SPL) [14, 15]. Similarly, freezers failed to activate premotor cortex and SPL during turning in a virtual reality environment [16]. Additionally, a decrease in resting state functional connectivity between the subthalamic nucleus and SMA [17], between the primary motor cortex (M1) and SMA, among frontoparietal regions and in the occipital cortex [18] have been found in FOG.

Studies of brain activity differences during upper limb movements are more scarce. Vercruysse *et al*. revealed hypo-activity in the dorsolateral prefrontal cortex, dorsal premotor cortex (dPMC) and M1, as well as hyper-activity in subcortical regions during continuous finger tapping in freezers compared to non-freezers [19]. Overall, these studies have been performed in patients while off medication, i.e. after at least 12 h of medication withdrawal. So far, no freezing-related studies have been conducted in functionally relevant motor tasks, such as writing. Additionally, there is a lack of studies performed while patients are on medication, reflecting the most common daily status. As mentioned above, even in this optimally medicated state and when controlling for disease confounds, difficulties in handwriting in freezers are more apparent when compared to non-freezers [7, 8]. In a visuospatial function test, more impairments were found in freezers than their non-freezing counterparts [20]. These results suggest a role for a dysfunctional dorsal visual stream in FOG, even when on medication.

The central research question of the current study is to gain more insight into the deficits freezers display in repetitive motor tasks other than walking. In line with the above-summarized findings, we hypothesize that freezers will display impairments in the visuomotor integration network, including visual, parietal and motor cortex, specifically involving the SMA, which will be correlated with more advanced micrographia. As cueing is an important compensatory strategy for patients with FOG, we also investigated the differential effects of visual cues on the neural network during handwriting in freezers and non-freezers. A case study demonstrated that the beneficial effects of visual cueing on FOG might be attributed to an increased information flow from visual and parietal areas to the motor cortex [21]. Given the positive effects of cueing in freezers, we hypothesize that connectivity in the network involved in external generation of movement (cued), involving visual, parietal, dorsal premotor and cerebellar areas, will be similar or even upregulated by adding visual cues in freezers.

METHODS

Subjects

Fifty-nine patients with PD were included, of which 42 were non-freezers and 17 were freezers. Behavioral performance of both groups have been described in detail elsewhere [7, 22]. Earlier work by our group also compared a partially overlapping non-freezer group with healthy controls at the neural network level (Nackaerts *et al.* – in revision). Freezers were identified according to item 1 of the New Freezing Of Gait Questionnaire (NFOG-Q) in which subjects indicated whether they had FOG in the past month after watching a video of various FOG episodes [23]. All freezers, except one, experienced freezing of gait both when on and off medication. Non-freezers never experienced FOG in the past month. Overall, 44 patients were tested in the current MRI study, 15 patients did not meet inclusion criteria for MRI (see below) or were not able to perform the task while lying down. Of the remaining 44 patients, five were excluded due to excessive head movements. In addition, activation levels did not pass the statistical threshold in one patient without freezing, even at uncorrected levels. Since a reliable identification of regions of interest (ROIs) at the single-subject level is an important requirement for Dynamic Causal Modeling (DCM), this patient was excluded. Finally, one patient was excluded as an influential outlier (> 2 standard deviations) at the behavioral level. For the main analysis, data of 37 patients with PD were included ($N_{non\text{-}freezers}$ = 27, $N_{freezers}$ = 10). As a recent review stressed the importance of accurately matching patient groups with and without FOG for disease severity [10], a sensitivity analysis was performed. For this purpose an independent researcher selected for each freezer a matched non-freezer based on the MDS-UPDRS-III

scores (N = 10 in each group). Hence, the current data are novel and present no overlap with previous work.

All participants were right-handed, as determined by the Edinburgh handedness scale [24]. Inclusion criteria consisted of: (i) diagnosis of PD according to the United Kingdom Parkinson's disease Society Brain Bank criteria [25]; (ii) Hoehn & Yahr (H&Y) stage I to III in the on-phase of the medication cycle, with the right side as disease dominant side in patients in H&Y I [26]; and (iii) clinical signs of micrographia as defined by a score of 1 or more on item II.7 of the Movement Disorder Society Unified Parkinson's Disease Rating Scale (MDS-UPDRS) regarding handwriting [27]. Exclusion criteria were: (i) Mini-Mental State Examination (MMSE) < 24 [28]; (ii) visual impairments that could not be corrected by glasses; (iii) upper limb problems other than PD that would impede handwriting; and (iv) contra-indications for MRI.

The study was approved by the local Ethics Committee of the University Hospitals Leuven in accordance with the code of Ethics of the World Medical Association (Declaration of Helsinki). Written informed consent was obtained prior to participation in the study and after explanation of the protocol.

Behavioral assessment

All participants underwent an extensive clinical test battery, including the MDS-UPDRS-III, H&Y staging scale as part of the MDS-UPDRS-III and NFOG-Q [23, 26, 27]. The Levodopa Equivalent Dose (LED) was calculated for each patient [29]. Fine motor skills were evaluated by means of the Manual Ability Measure (MAM-16) questionnaire [30] and emotional status was evaluated using the Hospital Anxiety and Depression Scale (HADS)[31]. As patients with FOG often experience more cognitive difficulties [32], we used the Montreal Cognitive Assessment (MoCA) to assess cognitive abilities [33, 34].

Writing performance outside the scanner was assessed using a touch-sensitive writing tablet [35]. Participants were instructed to produce a repetitive pre-writing task, by making three loops similar to the letter 'e' from the bottom of the blue to the top of the yellow target zone, corresponding to 0.6 cm (**Online Resource 1, Suppl. Fig. 1A**). After completion of the third loop, participants returned to the start circle via the gray zone. When re-entering the start circle, the loop-sequence disappeared from the screen allowing continuous repetition of the same figure without hand repositioning movements until the end of the 27 s trial [35]. The cued writing task was performed in the presence of the colored target zones. In the without-cue condition, the colored target zones disappeared after 1.5 s. The 'Systematic Screening of Handwriting Difficulties (SOS)' test, involving writing a text on paper for five minutes continuously, was used to assess daily life handwriting [36].

The above-described writing movement was also assessed in the scanner using a custom-made MRI-compatible touch-sensitive tablet (**Online Resource 1, Suppl. Fig. 1B**). The tablet was placed near the hip of the participant and adjusted in height and position to enhance writing comfort. Via a double mirror built into the head coil, real-time visual feedback of what was written was provided. A pacing tone was provided to standardize performance, i.e. participants were expected to complete one loop sequence in 2 s avoiding an auditory cue-effect on the individual up- and downstrokes. Both conditions (cued and uncued) were repeated four times within one run in random order. All participants performed three runs, with the exception of five patients of whom only two runs could be included due to excessive head movements. Before scanning, participants performed a practice session in a dummy scanner to become acquainted with the protocol. All handwriting (on tablet outside and inside the scanner and on the SOS-test) was performed with the right hand.

All testing occurred during the on-phase of the medication cycle, i.e. approximately one hour after last medication intake.

Processing and statistical analysis of handwriting performance

Data from the touch-sensitive tablet were filtered at 7 Hz with a 4^{th}-order Butterworth filter and processed using Matlab (R2011b). The primary outcome for writing inside and outside the scanner was writing amplitude (cm), defined by calculating local minima and maxima of individual up- and down-strokes. Additionally, variability in amplitude, i.e. the coefficient of variation (COV_{Ampl}, expressed as a percentage), and speed (cm/s) were determined. A blinded researcher evaluated the SOS-test manually. Mean writing size (mm) and writing velocity (letters written in five minutes) were determined. The total SOS-score, representing quality, was determined and consisted of: (i) fluency of letter formation; (ii) fluency in connections between letters; (iii) regularity of letter height; (iv) space between words; and (v) straightness of the sentences [36]. A higher total SOS-score indicated worse quality of handwriting (0-10).

Statistical analysis was performed using SPSS (version 24). Demographic characteristics and SOS outcomes were compared between patient groups using the Mann-Whitney U test and the Chi-squared test. A mixed model ANOVA was used to study differences between freezers and non-freezers on the writing tablet, with GROUP (non-freezer vs freezer) as a between-subject factor and CONDITION (cued-uncued) as a within-subject factor. MDS-UPDRS-III score and disease duration were included as covariates to control for between-group differences. In addition, a sensitivity analysis was conducted on subgroups stringently matched for disease severity using MDS-UPDRS-III scores (N = 10 in each group). A similar mixed model ANOVA was done, with LED included as covariate. The significance level for all tests was set at $\alpha < 0.05$.

Functional MRI acquisition and pre-processing

Imaging was carried out in a Philips Achieva 3T scanner. A standard head coil was used with foam padding to restrict head motion. High-resolution T1-weighted anatomical scans (T1 Turbo Field Echo (TFE) sequence, duration = 383 ms; slice number = 182; slice thickness = 1.2 mm; time repetition (TR) = 9.624 s; time echo (TE) = 4.6 ms; flip angle = 8°; matrix = 256 x 256; FOV = 218.4 x 250 x 250 mm) and T2*-weighted functional images were acquired for each participant using gradient echo-planar imaging (EPI) pulse sequence (50 transversal slices, slice thickness = 2.5 mm, slice gap = 0.25 mm, TE = 30 ms, TR = 3000 ms, flip angle = 90°, matrix = 80 × 80).

Functional imaging data were pre-processed using SPM8 implemented in Matlab (R2011a). All functional images were realigned to the reference (mean) image and co-registered to each subject's T1 anatomical image. All images were normalized to Montreal Neurological Institute (MNI) space and smoothed with a 6-mm full width at half maximum Gaussian kernel. Participants were excluded from further analysis in case of excessive head movement, determined by X, Y and Z-translations exceeding 2 mm or rotations (pitch, yaw and roll) of more than 2°. For both groups head motion parameters were assessed using the framewise displacement method [37], which revealed no differences between patients with and without FOG ($p = 0.539$).

Brain activity analysis

Data were analyzed using the general linear model approach in SPM8. The two experimental conditions (cued-uncued) were modeled and head motion parameters were added as covariates of no interest to correct for confounding effects induced by head movement. Basic main effects for both conditions were determined for each participant. Next, individual contrasts were entered into a second level ANOVA using a full factorial design. The main effects and interaction of GROUP (non-freezer vs freezer) and CONDITION (cued-uncued) were studied in a whole-brain analysis, with MDS-UPDRS-III score and disease duration as covariates. A similar analysis was also performed including only the matched groups, with LED as covariate.

Dynamic causal modeling

In the current study, differences in effective connectivity were investigated between freezers and non-freezers during visually cued and uncued handwriting using DCM, a Bayesian inference model [38]. We included ROIs known for their involvement in handwriting and visuomotor control [39, 40] and altered activation patterns in PD and specifically freezers [10, 41]. Hence, bilateral extrastriate visual cortex (MT/V5), bilateral SPL, left M1, left dPMC, left SMA and right cerebellar lobule VI were included. Next, the endogenous structure of the network (DCM-A) was defined based on previous studies of effective

connectivity of the visuomotor system [42-44]. Different models of varying complexity representing biologically plausible hypotheses on how connectivity might be modulated depending on the cueing-conditions (DCM-B) were constructed (**Online Resource 2, Suppl. Fig. 2**). The driving input (DCM-C) was set on MT/V5 across conditions and models, as this region is essential for processing visual information to guide movement [45], which is essential for handwriting. Finally, Bayesian model selection (BMS) was used to identify the model with the highest evidence, using a random effects approach [46], after which the coupling estimates of the winning model were extracted for each participant. For more information about the DCM-technique itself, the extraction of ROIs and BMS, we refer the reader to **Online Resource 2**.

Statistical analysis of connectivity data

A mixed model ANOVA was used to study differences between freezers and non-freezers in connection strengths, with GROUP (non-freezer vs freezer) as a between-subject factor and CONDITION (cued-uncued) and CONNECTION as within-subject factors. Only connections that survived a Bonferroni-corrected 1-sample t-test (accounting for the number of connections) for the entire group of participants were included (**Online resource 2, Suppl. Table II**). MDS-UPDRS-III scores and disease duration were added as covariates. Secondly, the matched sensitivity analysis was performed using the same approach, with LED as a covariate. For all analyses, a Greenhouse-Geisser correction was applied as the assumption of sphericity was violated. Finally, we performed an exploratory partial correlation analysis between coupling estimates of altered connections and performance measures on both the tablet and the SOS-test. Covariates added in the partial correlation analyses were the same as the ones for the mixed model ANOVAs. Statistical analysis was performed in SPSS (version 24) with a significance level of $\alpha < 0.05$.

RESULTS

Whole group analysis

Behavioral data

Demographics and clinical characteristics of freezers and non-freezers are described in **Table 1**. Freezers presented with a longer disease duration ($p = 0.009$) and higher MDS-UPDRS-III score ($p = 0.009$), suggesting worse disease severity.

Writing performance inside the scanner did not differ between groups or conditions. Outside the scanner, freezers showed a smaller writing size compared to non-freezers ($F_{(1, 33)} = 8.305$; $p = 0.007$; $\eta_p^2 = 0.201$; **Fig. 1A**). Also, there was a non-significant trend towards a GROUP x CONDITION interaction for writing speed ($F_{(1, 33)} = 3.867$; $p = 0.058$; $\eta_p^2 = 0.105$), with exploratory post hoc analysis showing that non-freezers

wrote more slowly during cued than uncued writing ($p < 0.001$), while no differences were found in freezers. COV_{Ampl} did not differ between groups or conditions. Finally, for writing during the SOS-test a trend towards a worse handwriting quality was found in freezers versus non-freezers ($p = 0.078$).

Table 1: General characteristics of the whole group analysis

	Freezers (N = 10)	Non-freezers (N = 27)	p-value
Age (years)	67.0 (62.3; 70.3)	63.0 (56.5; 69.0)	0.191
Gender (M / F)*	8 / 2	16 / 11	0.241
EHI (%)	100.0 (72.5; 100)	100.0 (90.0; 100.0)	0.724
HADS-Anxiety (0-21)	4.5 (3.0; 8.3)	5.0 (3.0; 8.5)	0.801
HADS-Depression (0-21)	6.5 (4.3; 7.0)	3.0 (1.5; 6.5)	0.121
MoCA (0-30)	27.0 (26.0; 28.0)	27.0 (25.0; 27.0)	0.353
H&Y (I / II / III)*	1 / 9 / 0	3 / 20 / 4	0.422
MDS-UPDRS-III (0-132)	37.0 (32.8; 43.5)	20.0 (16.0; 30.5)	**0.009**
Disease duration (years)	7.5 (5.3; 10.0)	4.0 (2.0; 7.0)	**0.009**
LED (mg/day)	530.8 (420.0; 653.8)	375.0 (180.0; 622.5)	0.158
NFOG-Q (0-23)	11.5 (6.5; 17.8)	-	-
MAM-16 (0-64	58.0 (57.0; 58.8)	58.0 (53.5; 60.5)	0.649

Median (first, third quartile) are displayed. **Abbreviations**: EHI = Edinburgh Handedness Inventory; HADS = Hospital Anxiety and Depression Scale; H&Y = Hoehn & Yahr stage; LED = Levodopa Equivalent Dose; MAM-16 = Manual Ability Measure; MDS-UPDRS-III = MDS Unified Parkinson's Disease Rating Scale part III; MoCA = Montreal Cognitive Assesment; NFOG-Q = New Freezing Of Gait Questionnaire.
* indicates variables analyzed using the Chi-squared test.

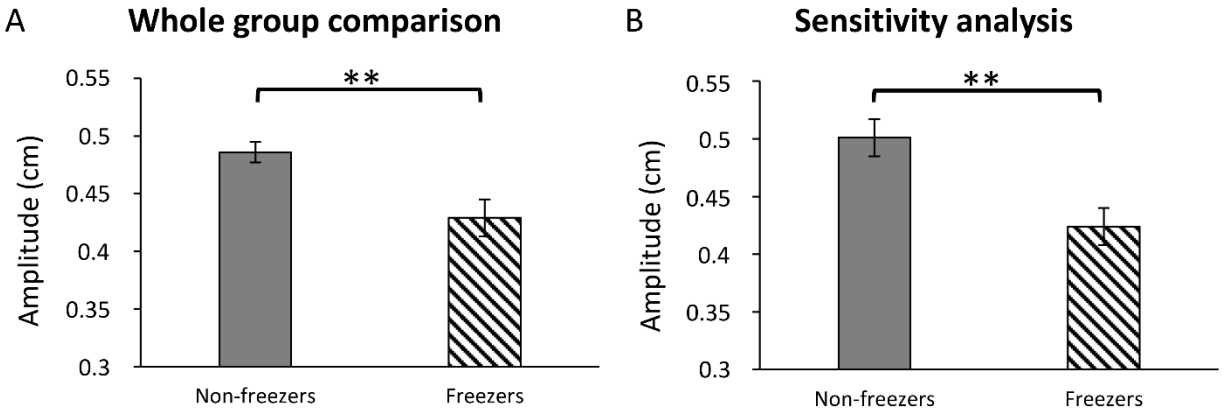

Figure 1: Behavioral results for writing amplitude outside the scanner (cm). (A) Whole group comparison (27 non-freezers vs 10 freezers). Values are corrected for MDS-UPDRS-III and disease duration; (B) Sensitivity analysis (10 non-freezers vs 10 freezers). Values are corrected for LED. ** $p < 0.01$; Error bars represent standard errors

Neural activation pattern

During writing, a network comprising bilateral MT/V5, bilateral SPL, left dPMC, left SMA, left M1 and right cerebellar lobule VI was activated across conditions in all participants (**Fig. 2**). There was a significant

increase in BOLD activity during cued compared to uncued writing in bilateral visual cortex ($p < 0.05$, family-wise error (FWE)-corrected). No differences between groups were found, nor any interaction.

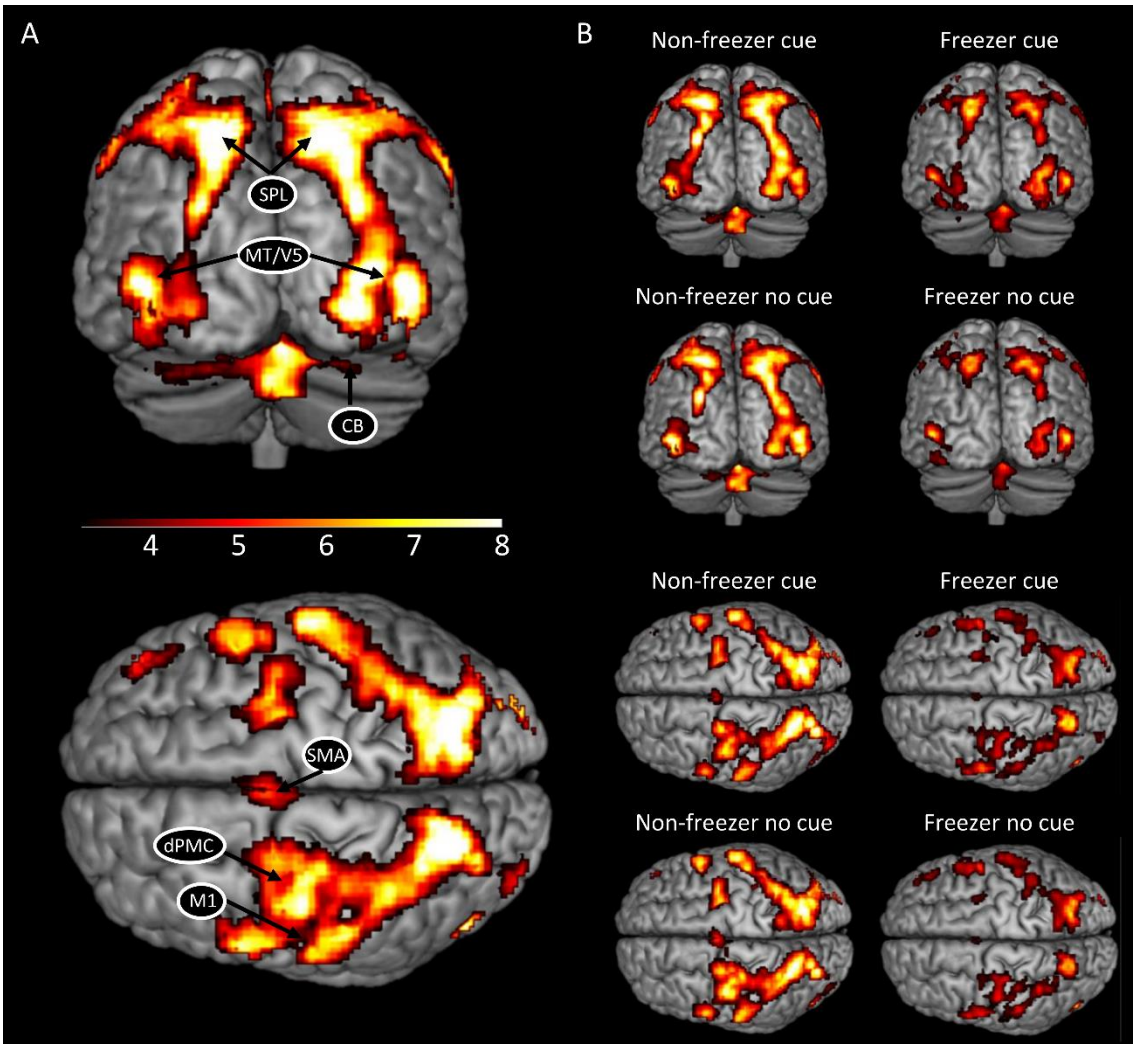

Figure 2: BOLD activation pattern during handwriting in the whole group comparison. (A) Activated network for both conditions combined; (B) Activated network in each condition and group separately. CB = cerebellum; dPMC = dorsal premotor cortex; M1 = primary motor cortex; SMA = supplementary motor area; SPL = superior parietal lobe; MT/V5 = motion sensitive middle temporal visual area. The threshold was set at $p < 0.001$ (uncorrected) to obtain better visualization of all areas

Connectivity analysis

The cueing-independent coupling (DCM-A), showed a strong tendency towards a GROUP x CONNECTION interaction ($F_{(18, 594)} = 2.372$; $p = 0.065$; $\eta_p^2 = 0.067$). Exploratory post hoc analysis revealed tendencies towards reduced coupling strength between left MT/V5 and left SPL ($p = 0.086$) and between left SPL and dPMC ($p = 0.065$) in freezers versus non-freezers, suggesting a reduced coupling in the left visuomotor integration network.

When comparing the cueing-dependent connectivity (DCM-B), we found a significant CUE x CONNECTION interaction ($F_{(18,\ 594)} = 2.390$; $p = 0.023$; $\eta_p^2 = 0.068$). In the presence of visual cues, coupling strength increased from right SPL, left dPMC and left SMA onto left SPL (all $p < 0.001$) and from left dPMC onto right SPL ($p = 0.036$) (**Fig. 3A**). In contrast, during uncued writing there was a stronger connectivity from left dPMC and left SMA to right cerebellum (resp. $p = 0.008$ and $p = 0.003$) and from left dPMC, left SMA and right cerebellum to left M1 (resp. $p = 0.006$, $p = 0.014$, $p = 0.040$) (**Fig. 3B**). Stronger inhibitory coupling was found bilaterally from SPL onto MT/V5 (left: $p = 0.023$ and right: $p = 0.003$) in the absence of cues. Hence, during cued writing connectivity targeting SPL was increased, while during uncued writing connectivity was enhanced in the (pre)motor-cerebellar loop. No differences were revealed between subgroups in these patterns.

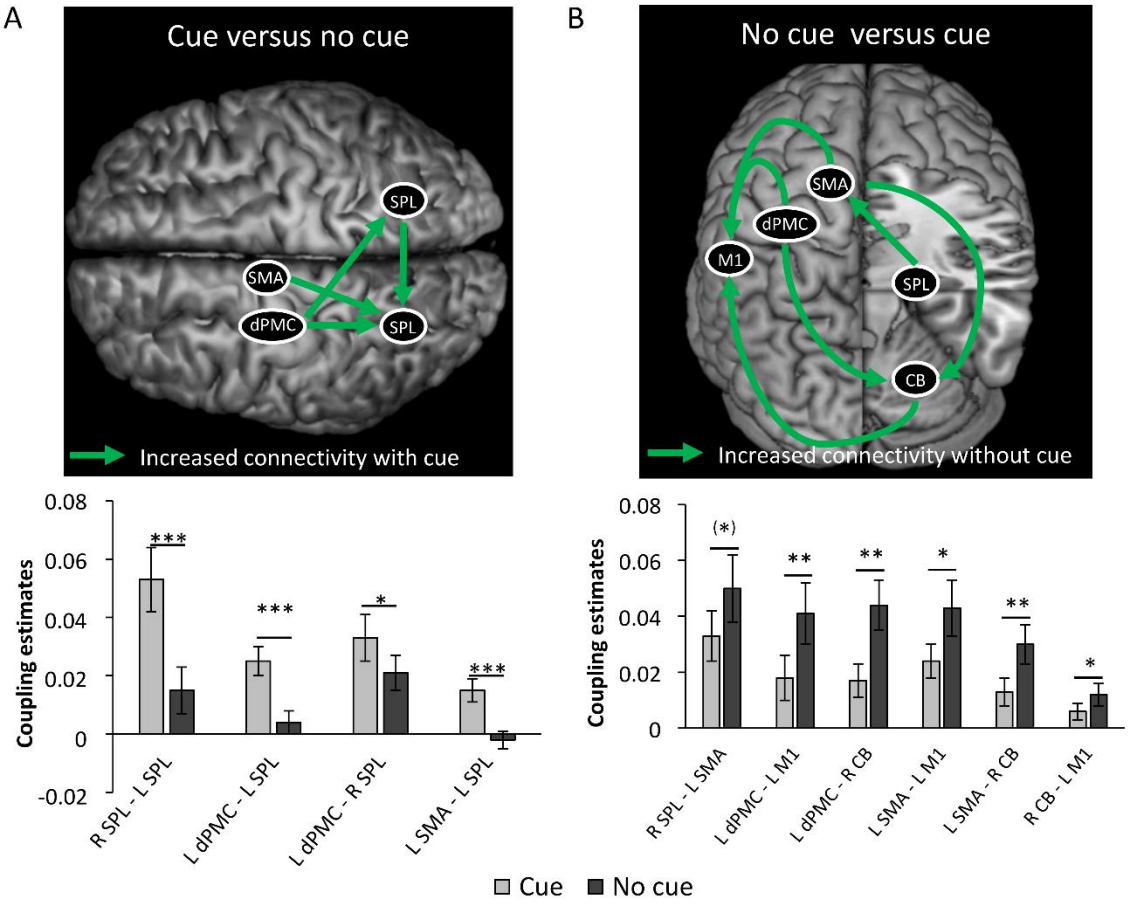

Figure 3: Difference in network connectivity between handwriting with and without cue (DCM-B) in the whole group comparison. (A) Increased connectivity with cue compared to without cue; (B) Increased connectivity without cue compared to with cue. Only excitatory connections are displayed, all corrected for MDS-UPDRS-III and disease duration. CB = cerebellum; dPMC = dorsal premotor cortex; M1 = primary motor cortex; SMA = supplementary motor area; SPL = superior parietal lobe; MT/V5 = motion sensitive middle temporal visual area. (*) $p < 0.1$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; Error bars represent standard errors

Sensitivity analysis

Behavioral data

Demographics and clinical characteristics of the well-matched groups are described in **Table 2**, though freezers had a higher dose of dopaminergic medication (p = 0.029).

Writing performance was similar to the one described for the whole group, with no differences in the scanner. Outside the scanner, freezers revealed a smaller writing size compared to non-freezers ($F_{(1, 17)}$ = 10.245; p = 0.005; $\eta_p^2 = 0.376$; **Fig. 1B**). In addition, amplitude was smaller during cued versus uncued writing ($F_{(1, 17)}$ = 5.938; p = 0.026; $\eta_p^2 = 0.259$). Finally, there was a GROUP x CONDITION interaction for writing speed ($F_{(1, 17)}$ = 5.867; p = 0.027; $\eta_p^2 = 0.257$), showing that the non-freezer group wrote more slowly during cued than uncued writing (p < 0.001), while no differences were found in the freezer group. No differences were found for COV_{Ampl} or on the SOS-test.

Table 2: General characteristics of the matched group analysis

	Freezers (N = 10)	Non-freezers (N = 10)	p-value
Age (years)	67.0 (62.3; 70.3)	64.0 (59.3; 71.0)	0.853
Gender (M / F)*	8 / 2	7 / 3	0.606
EHI (%)	100.0 (72.5; 100)	100.0 (90.0; 100.0)	0.796
HADS-Anxiety (0-21)	4.5 (3.0; 8.3)	8.0 (3.3; 8.8)	0.579
HADS-Despression (0-21)	6.5 (4.3; 7.0)	2.0 (1.0; 7.8)	0.393
MoCA (0-30)	27.0 (26.0; 28.0)	27.0 (25.0; 27.0)	0.165
H&Y (I / II / III)*	1 / 9 / 0	0 / 7 / 3	0.119
MDS-UPDRS-III (0-132)	37.0 (32.8; 43.5)	33.5 (29.8; 40.0)	0.579
Disease duration (years)	7.5 (5.3; 10.0)	4.5 (1.0; 7.8)	0.089
LED (mg/day)	530.8 (420.0; 653.8)	285.0 (105.0; 543.8)	**0.029**
NFOG-Q (0-23)	11.5 (6.5; 17.8)	-	-
MAM-16 (0-64	58.0 (57.0; 58.8)	56.0 (50.3; 58.0)	0.529

Median (first, third quartile) are displayed. **Abbreviations**: EHI = Edinburgh Handedness Inventory; HADS = Hospital Anxiety and Depression Scale; H&Y = Hoehn & Yahr stage; LED = Levodopa Equivalent Dose; MAM-16 = Manual Ability Measure; MDS-UPDRS-III = MDS Unified Parkinson's Disease Rating Scale part III; MoCA = Montreal Cognitive Assesment; NFOG-Q = New Freezing Of Gait Questionnaire.
* indicates variables analyzed using the Chi-squared test.

Neural activation pattern

A similar network to the one described for the whole-group analysis was activated. Also, during cued writing the bilateral visual cortex was activated more strongly compared to uncued writing (p < 0.05, FWE-corrected). No main effects of group or interactions were detected.

Connectivity analysis

The cueing-independent connectivity estimates (DCM-A) showed a significant GROUP x CONNECTION interaction ($F_{(18, 306)}$ = 3.031; p = 0.035; $\eta_p^2 = 0.151$). Group differences were driven by a weaker coupling strength in freezers from left MT/V5 to left SPL (p = 0.012), from left SPL to right SPL, left dPMC and left SMA (resp. p = 0.018, p = 0.013 and p = 0.013) and from left dPMC to left M1 (p = 0.022) (**Fig. 4**).

The cueing-dependent connectivity estimates (DCM-B) were not significantly different.

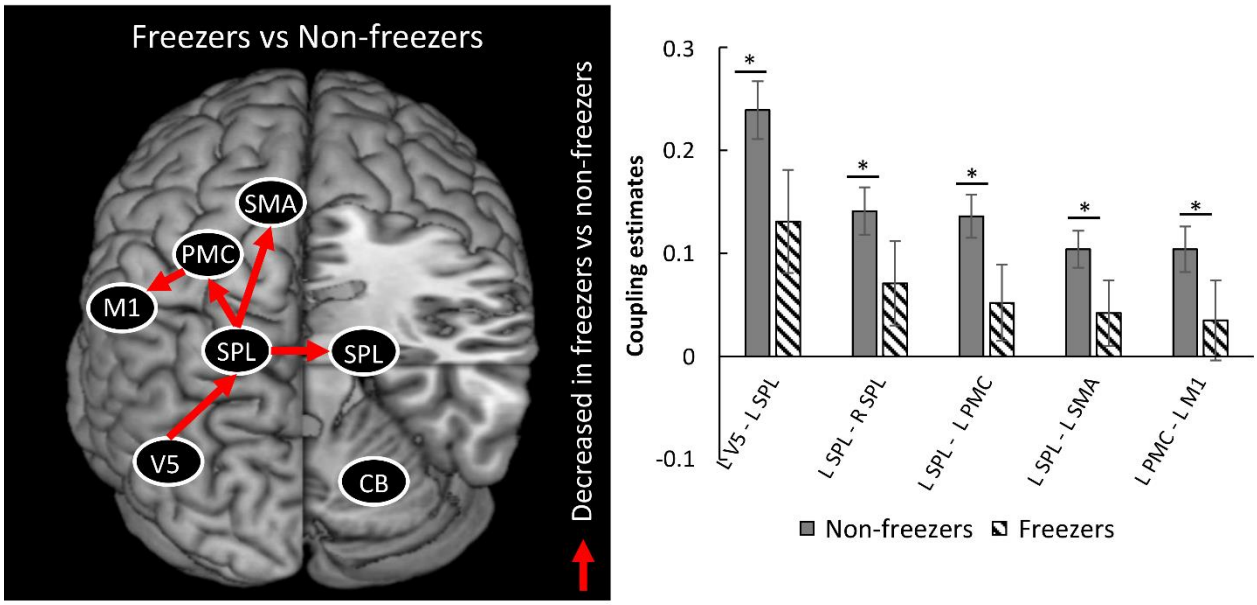

Figure 4: Difference in network connectivity between freezers and non-freezers during handwriting (DCM-A) in the sensitivity analysis. Only excitatory connections are displayed, all corrected for LED. CB = cerebellum; dPMC = dorsal premotor cortex; M1 = primary motor cortex; SMA = supplementary motor area; SPL = superior parietal lobe; MT/V5 = motion sensitive middle temporal visual area. * p < 0.05; Error bars represent standard errors

Correlation analysis

Exploratory partial correlation analysis revealed significant correlations between writing performance and effective connectivity in both patient groups. In non-freezers, a stronger connection from left MT/V5 to SPL correlated with slower handwriting outside the scanner (r = -0.693, p = 0.039). In addition, stronger coupling between left MT/V5 and SPL (r = -0.667, p = 0.050; **Fig. 5, upper left**) and between left SPL and dPMC (r = -0.838, p = 0.005) related to a better handwriting quality on the SOS-test. Finally, there was a correlation between increased connection strength from left SPL to left SMA (r = 0.691, p = 0.039; **Fig. 5, upper middle**), from left SPL to dPMC (r = 0.656, p = 0.055) and from left dPMC to M1 (r = 0.666, p = 0.050; **Fig. 5, upper right**) and a larger writing size on the SOS-test.

In freezers, stronger coupling between left SPL and dPMC correlated with larger (r = 0.677, p = 0.045) and slower handwriting in the scanner (r = -0.776, p = 0.014) and outside the scanner (r = -0.670, p = 0.048). In

addition, stronger coupling between left MT/V5 and SPL related to a better handwriting quality on the SOS-test (r = -0.707, p = 0.033) (**Fig. 5, lower left**). Finally, there was a correlation between increased connection strength from left dPMC to M1 and a larger writing size on the SOS-test (r = 0.676, p = 0.045) (**Fig. 5, lower right**). Contrary to non-freezers, there was no correlation between the connection from left SPL to SMA and writing size on the SOS-test (**Fig. 5, lower middle**).

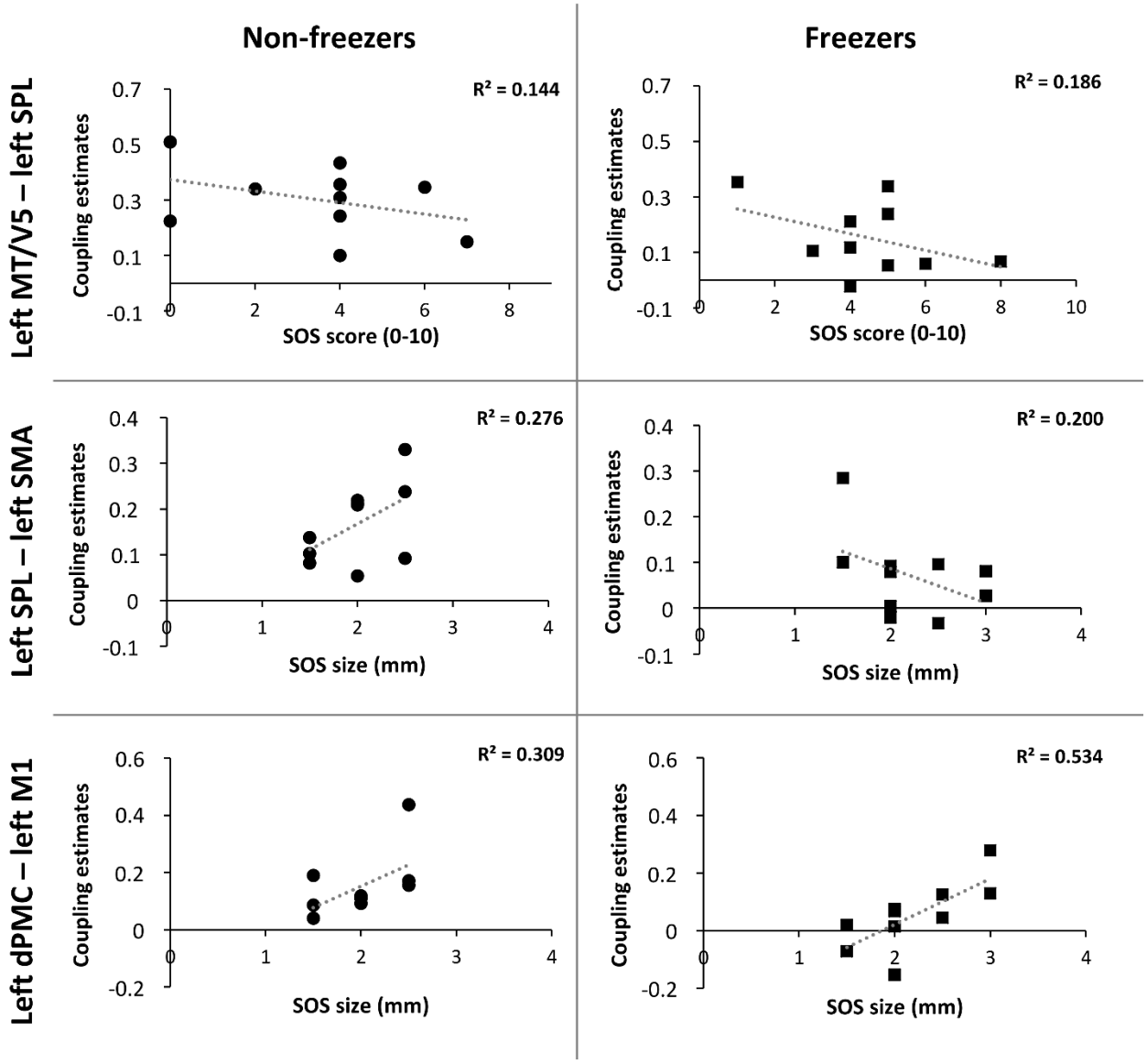

Figure 5: Correlations between writing performance on the SOS-test and coupling estimates. Left panels represent correlations of the non-freezer group, right panels of the freezer group. The dotted lines represent trend lines.

DISCUSSION

In this study, we showed reduced effective connectivity in the visuomotor integration network in freezers compared to non-freezers during handwriting, irrespective of cues. In line with our hypothesis, the correlation between coupling parameters and writing performance suggests that these connectivity alterations contribute to the aggravated micrographia, observed in the freezing cohort. This pattern of results was not significant in the whole group, but rigorous matching for disease severity further revealed the robustness of these effects. In addition, this extra analysis exposed additional connectivity impairments throughout the extended motor system in freezers, stressing the importance of accurately matching freezer and non-freezer subgroups [10]. In line with our second hypothesis and results from the behavioral domain, we also found that freezers and non-freezers were equally able to generate network coupling inherent to cued movement generation.

A recent review by Peterson *et al*. highlighted the role of cognition, specifically attention, executive function and visuospatial function, for mobility in FOG [47]. The important role of the visual system is emphasized by the fact that FOG episodes can be triggered by walking through small doorways [48, 49] or turning when the direction of gaze is shifting [50]. This role was recently supported in a study revealing increased activity in the visual cortex during turning in freezers [16]. Several other imaging studies also revealed altered visual networks in patients with freezing [18, 51, 52]. As previously suggested by Lord *et al*. [20], we found a dysfunctional dorsal visual stream in the left hemisphere in freezers during handwriting in general. We interpret this impairment of appropriate visuomotor integration as underlying parkinsonian handwriting deficits, since better coupling in this circuitry was correlated to better handwriting quality in both cohorts. The most specific difference between freezers and non-freezers was the reduced influence of the left SPL onto the SMA present in freezers. Moreover, in non-freezers a stronger input from the left SPL to the SMA correlated with a larger writing size on the SOS-test, suggesting a role for the SMA in amplitude regulation. The importance of the SMA in amplitude regulation has been suggested previously, as stimulation of the SMA using short-term repetitive transcranial magnetic stimulation resulted in increases in writing amplitude in patients with PD [53]. Additionally, Snijders *et al*. related decreased SMA activity to deficient regulation of step amplitude during imagery of gait in patients with FOG [15]. The current study extends this to handwriting, as results suggest that the weakened input to the SMA is an important contributor to the exaggerated micrographia observed in freezers, potentially leading to episodic amplitude dysregulation. We regard this result as freezing-specific, as we ruled out selection bias by performing one-to-one matching.

Furthermore, we detected decreased coupling in parietal-premotor and premotor-M1 connections in patients with FOG during handwriting. These parietal-premotor circuitries are often described as alternative networks in PD to compensate for the defective basal ganglia-motor loops [54-57]. These results may imply an insufficient cueing-independent compensatory mechanism in patients with freezing, and is reinforced by the finding that weaker dPMC-related connectivity correlated with decreased handwriting amplitude and quality in both groups. Additionally, Vercruysse *et al*. also showed decreased BOLD-activation in dPMC, M1 and dorsolateral prefrontal cortex in patients with FOG during continuous tapping [19].

Contrary to our hypothesis and the case study by Velu *et al*. [21], we did not find an upregulation in the visuo-parietal connection in response to visual cueing specifically in freezers. Rather, both freezers and non-freezers had a similar response and changed to the same networks for internal (uncued) and external (cued) motor control. In general, we saw an increase in parietal processing in the presence of visual cues, while during uncued writing there was an enhanced coupling between parietal and supplementary motor areas in combination with a greater involvement of the cerebellum and dPMC. Overall, these differences are consistent with the previously reported data on various other motor tasks with and without cues in both healthy subjects and patients with PD [41, 45, 58, 59]. The lack of an upregulated visuo-parietal coupling in freezers may be the result of the type of cues, which worked as an accuracy constraint rather than allowing either group to use the cues to their advantage. This is supported by the similar behavioral responses of both groups. In contrast to the whole group results, the sensitivity analysis did not reveal differences in connectivity between cued and uncued writing. It is possible that the networks involved in external generation of movement became more blurred in both groups, due to their matched and more severe disease profiles than apparent in the whole group. This is in line with findings in healthy elderly in whom fading of the uncued network was found compared to young adults, resulting in an overlap between networks involved in internal and external control of movements [60].

Writing while lying supine in the scanner was experienced as more difficult by patients, underscored by the fact that seven patients could not perform the task in the scanner. As such, the remaining sample size was relatively small, especially in the sensitivity analysis. Furthermore, LED doses differed in the sensitivity analysis, with freezers presenting with a higher LED in line with other studies [19, 20, 61]. We corrected for this difference by adding the LED as covariate, which did not influence our findings. In addition, the current study focused on patients with a mild to moderate disease severity, limiting the generalizability of the findings. Finally, during scanning there was no difference between patient groups at the behavioral level. However, similar to what was proposed for the comparison of patient groups and healthy controls

[62], it is important to match behavioral performance to allow a meaningful comparison at the neural level.

Conclusion

The current study provides new insights into the neural underpinnings of micrographia in PD patients with freezing mainly at the cortical level. Altered network interactions were found in PD patients with FOG during writing, suggesting weaker neural coupling in the left visuomotor processing network in freezers compared to non-freezers. Correlation analyses confirmed that a combination of impaired amplitude regulation and weakened supportive strategies contributed to micrographia in patients with PD and FOG. Finally, this study demonstrated that freezers activated similar cued and uncued networks as non-freezers.

CONFLICT OF INTEREST STATEMENT

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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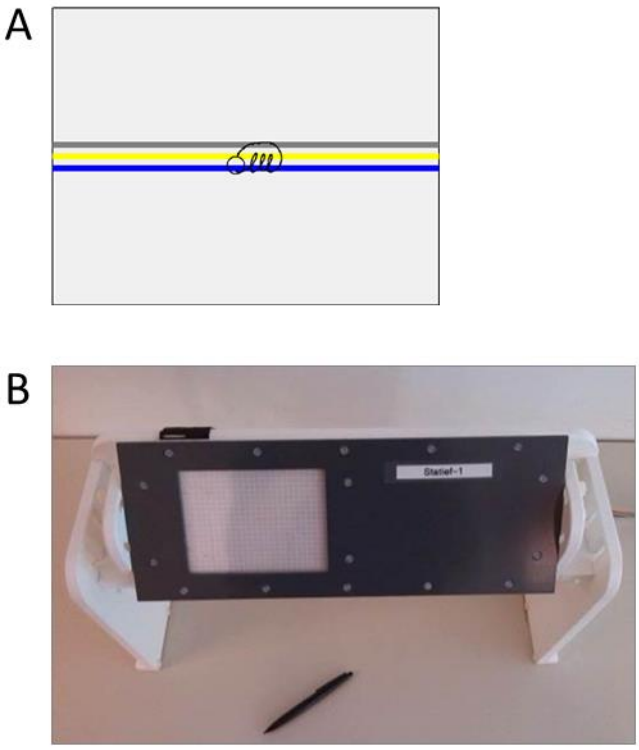
ONLINE RESOURCE 1



Supplementary Figure 1: Measurement equipment and writing tasks.

ONLINE RESOURCE 2

Dynamic causal modeling

Dynamic causal modeling (DCM) is a Bayesian inference method to model the influence that one neuronal system exerts over another (Friston *et al.*, 2003), measuring the directed coupling among neuronal responses (i.e. effective connectivity), rather than measuring dependencies among Blood Oxygen Level Dependent (BOLD) signals (i.e. functional connectivity). The method relies on *a priori* defined hypothesis-driven neuronal models of interacting brain regions relevant to a specific task.

DCMs are computed at the single-subject level. Therefore, we extracted the first eigenvariate of the BOLD time-series, adjusted for effects of interest from the eight ROIs at subject-specific coordinates. ROIs were defined as spheres (4 mm radius) centered upon individual activation maxima based on individually normalized SPMs (threshold $p < 0.001$; in case of non-significant voxels, the threshold was lowered to $p < 0.05$) (**Suppl.Table I**).

As mentioned in the main text, the endogenous structure of the network (DCM-A) was based on previous studies on effective connectivity of the extended motor system and alternative models on how connectivity might be modulated depending on the experimental conditions (DCM-B) were constructed (**Suppl. Fig.2**). The driving input, i.e. the influence of direct inputs to the system (DCM-C), was set at MT/V5. Bayesian model selection (BMS) was used to identify the model with the highest evidence, using a random effects approach. We identified the most likely model for the NFOG-FOG comparison by taking into account the exceedance probability for the model-set, i.e. the probability that a model is more likely to have generated the observed BOLD signal than any other model. Following BMS, the coupling estimates of the winning model were extracted for each participant.

Bayesian model selection

Ten different models were compared in a random-effects BMS. Model 1 was revealed as the winning model for both non-freezers and freezers, with an exceedance probability of resp. 99.9% and 84.9% (**Suppl. Fig. 3A, B & D**). Similarly, model 1 was the winning model for both patient groups in the sensitivity analysis, with an exceedance probability of 83.2% in the matched non-freezer group (**Suppl. Fig. 3C-D**).

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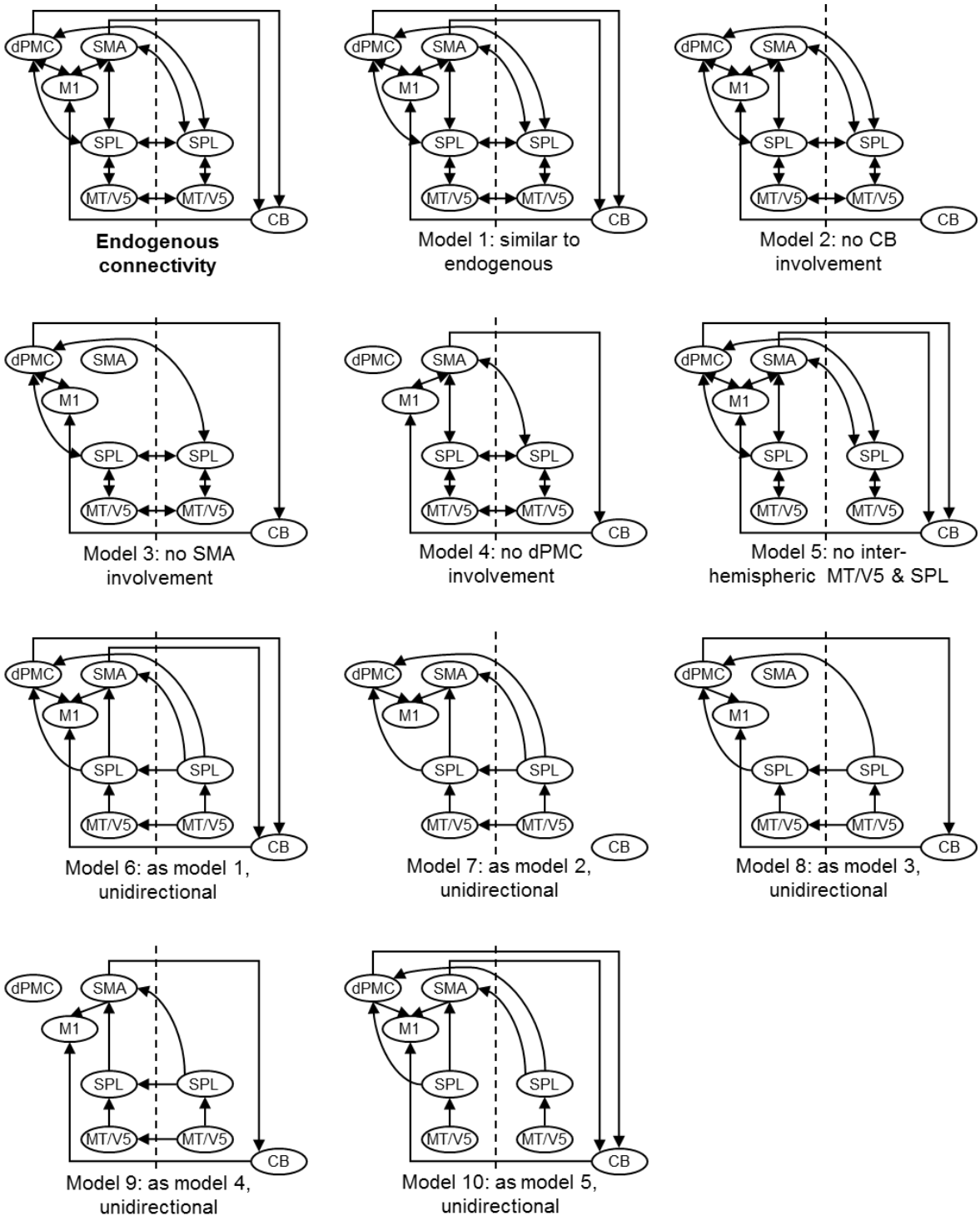
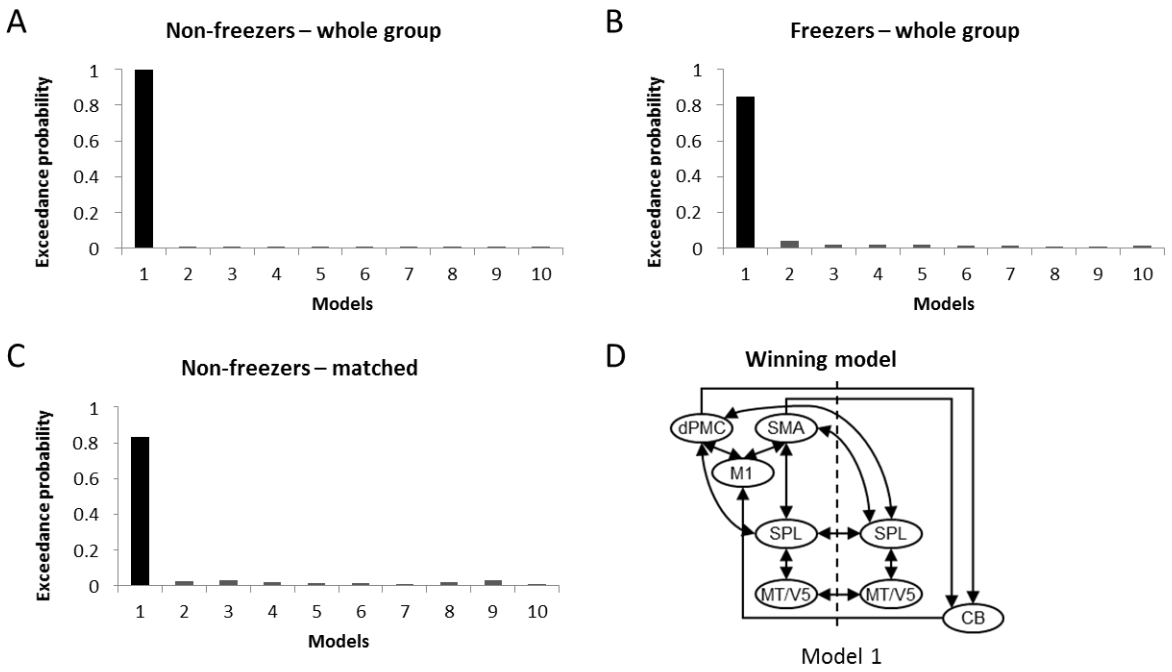


Supplementary figure 2: Ten models compared using Bayesian Model Selection. Model 1-10 represent modulations of the connections (DCM-B). The input was set at bilateral MT/V5. Abbreviations: CB = cerebellum; dPMC = dorsal premotor cortex; M1 = primary motor cortex; SMA = supplementary motor area; SPL = superior parietal lobe; MT/V5 = motion sensitive Middle Temporal visual area.



Supplementary Figure 3: Bayesian model selection. (A) Comparison of 10 models for non-freezers in the whole group comparison; (B) Comparison of 10 models for freezers in the whole group comparison; (C) Comparison of 10 models for non-freezers in the sensitivity analysis; (D) Winning model for the different comparisons.

Supplementary Table I: ROI coordinates for DCM analysis

	Freezers (N = 10)			Non-freezers (N = 27)		
	X	**Y**	**Z**	**X**	**Y**	**Z**
L M1	-36.0 ± 2.4	-26.8 ± 2.3	62.4 ± 3.6	-35.7 ± 4.6	-25.8 ± 3.7	61.3 ± 4.2
L dPMC	-26.4 ± 2.1	-9.8 ± 3.2	54.8 ± 3.0	-23.9 ± 2.2	-8.0 ± 3.7	55.3 ± 4.3
L SMA	-5.2 ± 1.5	-5.8 ± 2.6	61.6 ± 3.0	-5.8 ± 1.4	-5.8 ± 3.7	61.0 ± 5.7
L SPL	-25.2 ± 4.9	60.0 ± 3.9	59.8 ± 2.9	-23.8 ± 4.2	-60.4 ± 3.3	60.4 ± 3.4
R SPL	21.2 ± 3.7	-65.4 ± 3.9	58.0 ± 2.7	21.7 ± 4.0	-64.5 ± 4.2	59.6 ± 3.5
L MT/V5	-47.6 ± 2.3	-74.8 ± 3.0	1.0 ± 4.9	-46.8 ± 3.4	-72.3 ± 4.7	1.8 ± 4.6
R MT/V5	48.2 ± 2.5	-67.8 ± 3.5	-0.4 ± 4.6	48.0 ± 3.5	-67.7 ± 3.7	2.3 ± 3.5
R CB	29.2 ± 2.9	-51.8 ± 3.2	-28.8 ± 3.0	28.2 ± 3.7	-50.5 ± 4.2	-26.4 ± 3.3

The group coordinates are presented as mean ± standard deviation.
Abbreviations: CB = cerebellum; dPMC = dorsal Premotor cortex; L = left; M1 = primary motor cortex; R = right; SMA = Supplementary Motor Area; SPL = Superior Parietal Lobe; MT/V5 = motion sensitive Middle Temporal visual area.

Supplementary table II: connections that survived the Bonferroni corrected 1-sample t-test				
	Whole group analysis		Matched group analysis	
Connections	**DCM-A**	**DCM-B**	**DCM-A**	**DCM-B**
L MT/V5 – R MT/V5	Included	Included	Included	NS
L MT/V5 – L SPL	Included	NS	Included	NS
R MT/V5 – L MT/V5	Included	NS	Included	NS
R MT/V5 – R SPL	Included	Included	Included	Included
L SPL – L MT/V5	NS	Included	NS	Included
L SPL – R SPL	Included	Included	Included	Included
L SPL – L dPMC	Included	Included	Included	Included
L SPL – L SMA	Included	Included	Included	Included
R SPL – R MT/V5	NS	Included	NS	Included
R SPL – L SPL	Included	Included	Included	Included
R SPL – L dPMC	Included	Included	Included	Included
R SPL – L SMA	Included	Included	Included	Included
L dPMC – L SPL	Included	Included	Included	Included
L dPMC – R SPL	Included	Included	Included	NS
L dPMC – L M1	Included	Included	Included	Included
L dPMC – R CB	Included	Included	Included	Included
L SMA – L SPL	Included	Included	Included	NS
L SMA – R SPL	Included	Included	Included	NS
L SMA – L M1	Included	Included	Included	Included
L SMA – R CB	Included	Included	Included	Included
L M1 – L dPMC	NS	NS	NS	NS
L M1 – L SMA	NS	NS	NS	NS
R CB – L M1	Included	Included	Included	NS

Abbreviations: CB = cerebellum; dPMC = dorsal Premotor cortex; EXP = experimental writing training; HC = healthy controls; L = left; M1 = primary motor cortex; MT/V5 = extrastriate visual cortex; NS = not significant; R = right; PD = Parkinson's disease; PLB = placebo training; SMA = Supplementary Motor Area; SPL = Superior Parietal Lobe